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COCCUS PYOGENES AUREUS.

BY

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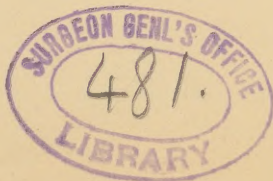
**THE TOXINES PRODUCED BY THE STAPHYLO-
COCCUS PYOGENES AUREUS.**

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THE following is a report of the results of an investigation of the metabolic products formed by the staphylococcus pyogenes aureus carried on in the Biological Laboratory of the University of Cincinnati.

Two cultures were used ; one kindly furnished me by Dr. Novy, of Ann Arbor, recently obtained from the blood of a child that had died of pyemia ; the other a culture of two years' standing. No appreciable difference in result was recognized. A bouillon containing 1 per cent. of meat extract and 1 per cent. of peptone was used as a culture-medium. The germs were grown in Erlenmeyer flasks containing from 40 to 50 c.c. After remaining in the incubator at a temperature of from 35° to 37° C. for seven days, the cultures were passed through Chamberland filters and the sterility of the filtrate established both by microscopic examination and inoculation upon agar.

This filtrate injected into rabbits produced no apparent symptoms. Thus, a rabbit weighing two pounds received 8 c.c. without effect. A daily



injection of 2 c.c. during a period of seven days gave a similar result. This result was to be expected in view of the well-known resistance of the rabbit to this microorganism. The injection of four centigrams of the filtrate in the left forearm of the author was followed by marked local and general reaction. Five hours after the injection the temperature had risen from 98.4° to 99.8° F., pulse 96, accompanied by headache, pain in the back, flushing of the face, and general malaise. Locally, the point of injection was red, tender, and showed slight edema. Within the next eight hours the temperature returned to normal, some headache and malaise remaining during the succeeding twelve hours. The local inflammation increased during this time, but subsided completely after seventy-two hours, and was followed by slight desquamation. Another injection of 6 c.c. was followed by essentially the same symptoms, though to a less degree, a rise of temperature from 98.5° to 99° , pulse 90, slight headache, and a marked local inflammation.

From these experiments the conclusion was reached that the staphylococcus produced in its culture one or more chemical compounds which, when injected, gave rise to characteristic general symptoms and marked local irritation.

The next step in the investigation was the determination of the properties of these compounds, and, if possible, their location. When subjected to a temperature of 60° C. for half an hour the filtrate produced no general symptoms, even when large doses were injected. The local effect, however, remained unchanged. A quantity of filtrate was

added to ten times its volume of absolute alcohol. A grayish-white precipitate resulted. This was allowed to settle to the bottom, the supernatant alcohol drawn off, and the precipitate repeatedly washed with 90 per cent. alcohol. After purification in this way the precipitate was dried in a vacuum at 33° C., and a light tan-colored powder was obtained.

This powder proved to be only partly soluble in water, but completely soluble in a 1 per cent. solution of sodium carbonate. A portion of this powder was then rubbed up with water and the insoluble portion separated by filtration. The filtrate was then thrown into ten volumes of alcohol; the resulting precipitate was re-dissolved and again precipitated with alcohol. After repeating this operation six times, and drying in a vacuum, a light grayish-white powder was obtained, albuminous in character, which, when injected, produced a very slight local and no general reaction. Another portion of the original powder was dissolved in a 1 per cent. solution of sodium carbonate. The injection of this in very small doses, *i. e.* (1 mg.) produced a very marked local irritation.

In all these experiments control-injections of bouillon were made, with negative results.

The insoluble alcoholic precipitate gave the following chemical reactions:

The light, tan-colored powder was incompletely soluble in water and dilute saline solutions. When water was added the powder became a seal-brown flocculent mass. It was completely soluble in a 1

per cent. solution of sodium carbonate; insoluble in alcohol and ether.

It was precipitated from its solution by alcohol, mercuric chloride, ammonium sulphate, and acetic acid.

It gave the biuret reaction, and with Millon's reagent behaved like a proteid.

It was not precipitated by heat, nitric acid and heat, sodium chloride, or sodium sulphate.

By virtue of these reactions the body is to be classed among the albumins. To which particular variety it belongs is not at present apparent.

As the result of this investigation the following conclusions may be drawn:

The staphylococcus pyogenes aureus produces toxic albuminous products of two kinds:

1. A readily diffusible substance, difficult of isolation, very susceptible to heat and chemical reagents, which, when absorbed in the human system, produces symptoms of general disturbance.

2. A less diffusible albuminous substance, not very susceptible to a temperature of from 60° to 70° C., or to chemical reagents, which, when injected in the human subject, produces at the point of injection a well-marked inflammation.

Christmas, working in Pasteur's laboratory, was the first investigator to take up this point. He obtained from bouillon-cultures of the staphylococcus pyogenes aureus, by the addition of alcohol, a precipitate that produced suppuration when injected in the anterior chamber of the eye and subcutaneous tissue of rabbits.

In the same way Brieger and Fraenkel obtained a

toxalbumin insoluble in water, which proved fatal when injected in rabbits or guinea-pigs. On post-mortem examination of such animals they found the following changes: Intense inflammation at the point of injection, with swelling and reddening of the neighboring tissues; at times a purulent necrosis at the point of injection, which in some cases extended through the abdominal wall and gave rise to an antiseptic purulent peritonitis. The amount of toxalbumin injected by these authors is not stated, but it must have been a very large quantity in order to produce such grave changes. I should judge that in these experiments the animals were killed by the violence of the local insult rather than by the absorption of any general toxic product.

My investigation would lead me to believe that the rabbit is proof against the general toxine produced by this germ, and that, moreover, the chemical operations employed in endeavoring to isolate it would destroy any toxic properties that it may have possessed.

There is one other point to which I desire to call attention—the therapeutic possibilities offered by these substances. If we take the sterile filtrate of staphylococcus cultures, and heat it for half an hour at 60° C., we have an irritant that I think will prove of considerable value. We have a counter-irritant that, when injected in very small doses, produces a well-marked irritation; in larger doses, almost a true erysipelalous inflammation. There are no dangers of general disturbance attending its use, there is no abrasion of the surface to become infected, and the effect to be produced is thoroughly within control.

If a $\frac{1}{2}$ per cent. of carbolic acid be added to it the fluid will keep indefinitely, or, better still, a 1 per cent. solution of the alcoholic precipitate in 1 per cent. sodium carbonate solution could be used. Such solutions would be of great value, both as counter-irritants and as a means of hastening suppuration in furuncles, etc.

If, on the results of these investigations, it is permissible to construct a theory of suppuration as produced by the staphylococcus, it would be briefly as follows: The staphylococcus, during its growth in the tissues, produces a toxine that, when absorbed, gives rise to the general symptoms attending a local suppurative process. Furthermore, it produces a toxic substance that is highly irritant, and that possesses a strongly positive chemotaxis for the leukocytes. The duty of the local leukocytosis is two-fold: First, by virtue of their phagocytic properties the leukocytes restrain the growth of the germ, and secondly, the local necrosis caused by their effusion enables the organism to discharge the bacteria, toxic products, and necrotic tissue present.

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